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DEVELOPMENT OF A ROBOTIC SYSTEM FOR THE ROUTINE ANALYSIS OF PESTICIDES IN BIOTA BY ACID DIGESTION AND SOLVENT EXTRACTION SOLVENT EXTRACTION

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INTRODUCTION

The increased public awareness of the presence of persistent toxic chemicals in the food chain has led to an increase in the number of analyses that must be performed annually. To meet this requirement, laboratories have to become more automated to deal with large number of repetitive samples with high priority. Laboratory automation in the past decade has primarily been focused at the sample analysis and data processing areas. Automated sample preparation and processing have not been addressed until recently. Since the sample preparation steps are usually time consuming, repetitive and prone to error, a robotic system in lieu of human operation is thus a logical choice.

This paper presents an overview on the development of a robotic workstation for the preparation of fish samples using acid digestion method. The system being used to implement this procedure is the Zymate II Laboratory Automation System, manufactured by Zymark Corporation of Hopkington, Mass.

The system design objectives are as follows:

i. Improved precision and accuracy of results.

ii. Cost savings in materials such as solvents.

iii. Reduced operator exposure to hazardous chemicals.

iv. Improved data reduction and reporting.

v. User friendliness.

The development of this system included the acquisition and modification of the hardware required by the analytical method, the establishment of the robotic procedures based on precise specifications of a manual method, the design and testing of software, and validation of the completed system.

SYSTEM HARDWARE

After evaluation of the manual method and the availability of robotic workstations, the following robotic components were selected:

i. Robot arm and Controller

ii. Power and Event Controller

iii. Robotic Hands (General Purpose, Blank and Cannular)

iv. Master Laboratory Station and Remote Pipetting Kit

v. Electronic Balance

vi. Sample Conditioning Station (Linear Shaker)

vii. Capping Station

viii. Remote Computer Interface

ix. Centrifugation Station

x. High Volume Dispensing Kit

xi. Drying/Neutralization (Prep-Sep) Station

xii. 10-ml Pipette Kit

xiii. Waste/Rinse Station

xiv. Cannular Wash Station

xv. Assorted Racks for Centrifuge Tubes

xvi. Purge Kit

xvii. Printer

Contract specifications also required that all sample attributes be stored on a remote PC and that a report be generated for each sample. To fulfill this requirement a microcomputer (IBM compatible) with 20 MB hard-drive, floppy disc drive and two serial ports were obtained and interfaced to the system.

Software used in the development consisted of Zymark's EasyLab language for robot controller programs and GW-BASIC running under DOS on the PC. Selection of the Basic language was influenced by the fact that most users are familia with it, and since it is an interpreted rather than compiled language, it wil

consequently speed software development.

DESCRIPTION OF MAJOR WORKSTATION COMPONENTS

a) Robot Module

The "robot" itself consists of an "arm" secured to a benchtop by a pedestal. This allows three degrees of freedom of motion. The arm is capable of holding a variety of "hands", which rotate about a wrist connection, giving one extra degree of freedom.

The range of motion in each direction is:

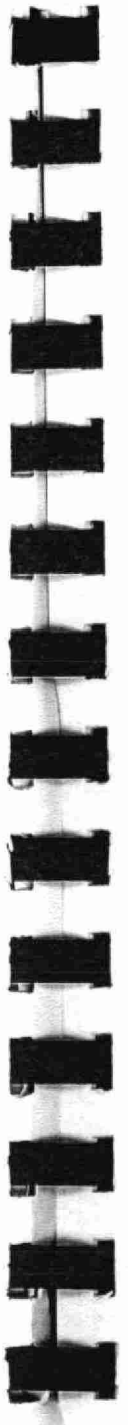
(a) Vertical : 0-34 cm
(b) Reach : 0-32 cm
(c) Rotary : 0-376^0

The arm's locomotive power is provided by a wire pulley system, driven by DC servomotors. Directional control is provided by programmed commands. Initial calibration of the arm at the factory provides a frame of reference, so the arm "knows" it's current position. To go to a new position, the controller compares the current position to the desired position. This generates an error signal, which drives the servo's in such a way as to minimize the error signal.

There are four different types of hands available to this system:

(a) General Purpose Hand
(b) Syringe Hand
(c) Dual Function
(d) Blank Hand

In this application, the General Purpose Hand, the Blank Hand and the Cannular Hand are used. The General Purpose Hand consists of a Teflon block housing a servomotor and has two "fingers" (grippers) attached. This hand is used for manipulation of glassware. The joint of the hand and arm forms a "wrist" capable of rotation form -5 to 365^0. In addition, the force with which the fingers grip an object can be adjusted from 0 to 200 units.



The arm and hand assemblies can also receive feedback from the external world in each of three axis:

ie: Vertical Force
Reach Force
Rotary Force

as well as Grip Force in the hand. This allows the arm to "sense" possible error conditions while performing operations on the bench.

The arm also allows different speed settings for each axis and can sense a collision, in which case it stops, and can only be restarted by the operator intervention.

b) Master Laboratory Station and Remote Pipetting Kit

(i) The Master Laboratory Station

This station consists of a housing containing three 10ml syringes valves to control fluid flow, stepper motors to drive the plungers and a dispenser nozzle mounted on a post.

The syringes can be programmed either independantly or jointly, t dispense/fill from a reservoir. Plunger speed can also vary t compensate for fluids of different viscosities.

The Dispenser nozzle contains separate outlets to avoid cros contamination of liquids. The syringes are programmed by setting th volume, speed and flow conditions necessary in a given operation.

Accuracy and precision for this station are quoted at 0.5% and 0.2% respectively, where volumes are greater than 20% of total syring volume.

(ii) Remote Pipetting Kit

The remote Pipetting Kit allows pipetting/dispensing to vessels a fixed positions on the benchtop. It consists of a Blank Hand with pipet-tip holder and pneumatic shucker, the parking station for thi

hand, and tubing for attachment to external compressed air, the MLS, solenoid operated valve, and a pipet-tip rack.

After attachment of this hand, programming is the same as for programming of any other robot moves. The pipet-shucking operation is driven from the Power and Event Controller, where actuation is by switch closure.

c) **Power and Event Controller**

The Power and Event Controller permits programmed operation of external devices. It provides the options listed in the manufacturer's documentation.

In this application, it is used to discard pipet tips and operate vacuum for the Rinse/Waste station. The large number of control functions will permit future expansion to the system, should it be required.

d) **Printer Module**

The system permits operation of a printer to provide hard copies of data and programs. It can be programmed to be either enabled or disabled and provides essentially a line by line screen dump.

e) **Balance Module**

The balance used in this application is a Mettler PE200. The module consists of the Balance Interface Card, a 5-pin to 32-pin cable and a sample tube holder. The balance is capable of sending weight results to the Controller via the balance interface card, which is compatible with Mettler's CL interface and capable of receiving commands from the

controller. It is a menu-driven progammable unit, where baud rate, parity and data transfer Mode may be set and stored. In addition, dampening functions may be set to compensate for vibration and time-averaged readings are also possible.

Operation is controlled by the Zymark controller, where weight and tare commands are defined. Results are stored in arrays for use in calculation of masses.

f) **Capping Station**

The Capping Station consists of the capper (an enclosed motor with a set of jaws on the top surface), and a cap parking station, where bottle caps are temporarily held.

The Capper's operation must be carefully coordinated with that of the arm. During a cap operation, a cap is retrieved from the parking station and lowered onto the tube while the capper rotates. Similarly, during an uncap operation, the tube is placed in the capper, the jaw grips the cap and the capper rotates in the opposite direction while the arm is incrementally raised.

The station can be programmed for capping torque and number of turns required to cap or uncap. Error routines are incorporated into the software to detect most common types of errors i.e. grip slipping etc.

g) **Drying/Neutralization Station**

This station consists of a prep-sep dispenser and a prep-sep workstation. It is run from the PEC, where a switch closure will result in pressure being applied to a prep-sep containing a solvent aliquot. The prep-seps contain a layer of sodium bicarbonate (0.5g)

and a layer of anhydrous sodium sulfate (0.5g). The pressure forces the solvent through the prep-sep cartridge, where the aliquot is dried and neutralized.

This workstation will save the expense of a powder pouring station and its associated equipment as well as bench space and time during the processing.

h) Sample Conditioning Station

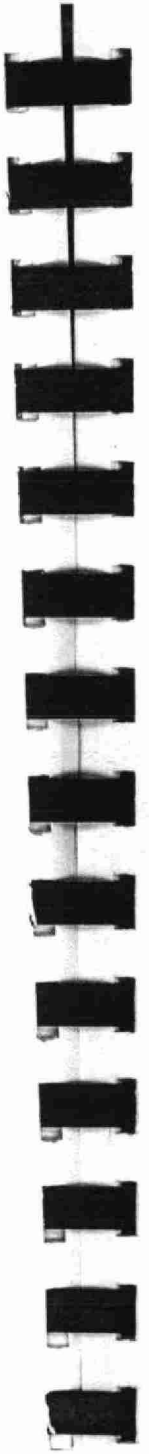
The Sample Conditioning Station consists of a linear shaker and two vertical, five position rack. The station is used for the agitation of samples during digestion and extraction, where the arm will load the tubes in a horizontal position.

The shaker is capable of speeds from 1 - 100 units and always stops at the same position, to faciliate access by the robot arm during insertion/removal from the rack.

i) Waste/Rinse Station

The Waste/Rinse Station will be used to aspirate the fish/acid residue and unused solvent to a waste holding tank. It is run from the PEC, where switch closure results in vacuum being applied.

The Rinse part of this station consists of a Hi-Volume Dispensing Kit, which contains a high-speed peristatic pump, tubing, valving, post and dispenser nozzles. When switched on, the tube will be washed out with water, supplied by the pump.



OUTLINE OF SYSTEM CONTROLLER

a) Hardware

The Zymate II System employes several 16-bit chips. The main CPU is t Intel 8088, with an 8087 used as a math co-processor (the Fast Math upgra from the Zymate I). Total memory is 64K, with the operating system occupyi 4K and leaving 60K for user applications. RAM in this system is non-volat and battery-supported.

The Zymark System is very flexible due to the modular concept. In t design, each module (ie Capper, MLS) has it's own module card, where i intelligence is contained on ROM chips. The module card is linked to i module by a control cable, and to the controller by module support boar Each module support board can hold up to five module cards, and there is ro for five module support boards, hence, we may have up to 25 modules in a giv application.

The System may be interfaced to an external computer by use of t Computer Interface Module. This module provides an RS232/423, ASCII, ser communications link between the controller and an external, more powerf laboratory computer. The interface parameters that may be set are:

(i) Baud Rate	: Up to 1200 Baud
(ii) Character Length	: 7 or 8 bits/character
(iii) Stop Bits	: one or two

This will allow the Computer Interface to communicate to almost any PC choose to use, providing it has a communication board and uses the same RS 2 standard interface.

b) Software

The Zymate II System provides a menu-driven system for operat convenience.

Upon initialization of the system, the Controller polls the modules plugged into the module support boards, and configures the system accordingly. The initial menu is displayed, and gives one five choices:

1. Run Program: allows one to execute an existing EasyLab Program.
2. Edit Program: allows one to edit or create programs. This is a line-editor, sufficient for most purposes.
3. Bench and Module Setup: this selection displays the modules currently configured in the system. From this menu, we may enter a control "page" which gives direct control over that particular module. Each module has a control page where defaults are set or changed. An example is the computer interface, where the baud rate, parity and stop bits may be set, depending on the characteristics of the external computer.
4. Systems Management: displays the status of the RAM batteries, amount of dictionary space left, and allows one to specify an autostart program.
5. Direct Control: allows the execution of programs and commands directly.

The Zymate II allows programs to be written in it's language called EasyLab. This is an interpreted language and has a flavor characteristic of BASIC. For example, the following constructs are allowed:

(i) Arrays: numeric, single dimension only.

(ii) DO Loops

(iii) GOTO

(iv) IF conditions THEN statements : but no ELSE construct.

(v) Real Math: 6 Significant figures.

(vi) Standard Mathematical Functions : SIN, COS, TAN, SQRT and LOG

EasyLab software allows any user-defined variables to be globally available to any sub-routine called from within any program. The maximum number of levels of calls is limited to 7. Text processing is very limited, as there is only one type of PRINT statement.

To overcome the programming limitations of Easy Lab and the Controller, an external computer was linked to the controller via the computer interface. With this configuration, a much more "intelligent" system is designed, where the external computer can be executing a more complex program, and simply pass the appropriate commands to the controller. Likewise, the controller can pass information back to the computer, where decisions will be made.

The choice of a high-level language running on the external computer is essentially wide open, but QUICK-BASIC, a compiled version of BASIC, was recommened.

CHANGE TO MANUAL PROCEDURE

In order to convert the manual extraction method to a robotic procedure the following modifications were made:

1. The original manual method requires extractions to be carried out in 60 ml centrifuge tubes. Since the capacity of the centrifuge module in the robotic system is for 50 ml tubes, all volumes were adjusted accordingly.
2. Robotic pipetting procedures were performed gravimetrically.
3. In the manual procedure, extract neutralization and drying steps were performed in Erlenmeyer flasks. To implement this step in the same manner would require too much bench space and additional glassware to be manipulated by the arm. In addition, the dispensing of powders are sufficiently time-consuming as to reduce sample throughput. Our solution was to proceed with disposable columns packed with sodium bicarbonate and anhydrous sodium sulfate, which required only one additional workstation.
4. The inclusion of a centrifuge module to break up emulsions and settle undigested fibrous material enabled better separation of phases.
5. The manual procedure required the final extract to be volumetrically diluted to 100.00 mls. in which a one-gram equivalent (or 1/5 of total volume) was removed for cleanup. Again, table space does not permit the required number of flasks. Instead, the total volume of extract was computed and a calculated volume was pipetted volumetrically.

DESCRIPTIONS OF ROBOTIC PROCEDURE

The final implementation of the manual procedure to a robotic procedure resulted in the following robotic method. This method is described as a linear version. Due to the sample throughput requirements, this linear procedure was serialized i.e. several processes and different samples are being processed concurrently on the table at the same time.

1. The initial step consists of taring a rack of 24 capped, empty centrifuge tubes, which will be loaded with samples. This step is performed independantly of the main run sequence. All weights are stored in an array.

2. After taring, the operator removes the rack of tubes and loads each with 5.0 ± 0.1 g of wet fish tissue. The tolerance of the weight must be adhered to, in order to ensure the centrifuge remains balanced. The loaded rack is replaced on the table and the run is initiated.

3. Operator enters run date, report header information, number of samples to be run, and sample attributes (sample code and type). A program is executed for all tubes where each tube is removed from the rack, loaded into the balance. The weight is stored in a data file and the tube is then unloaded from the balance and returned to the rack. This procedure is repeated for all tubes.

4. A sample tube is taken from the rack, and weighed to obtain actual sample weight.

5. When finished, the operator starts a digestion run. This consists of the following robotic steps:
   a) The loaded tube is removed from the rack.
   b) It is then weighed again on the balance
   c) A syringe on the Master Lab Station is prefilled with conc. HCl.
   d) The tube is uncapped and the cap parked.
   e) A 20 ml portion of acid is added to the sample.
   f) The tube is then capped.
   g) The tube is weighed.
   h) The tube which now contains the sample and acid is returned to the rack.
   i) This process is repeated sequentially for each tube.

6. At the meantime, samples are allowed to digest overnight. When the samples are digested, an extraction routine which consists of the following steps is executed.
   a) The tube containing the digested sample is removed from the rack.
   b) The arm changes it's grip on the tube by loading it into the capper and shifting it's grip so it grasps the tube by the middle. This is necessary due to the construction of the linear shaker.
   c) The tube is loaded into the shaker and allowed to agitate for 45 minutes.
   d) The tube is unloaded from the shaker, uncapped, 20 mls of extraction solvent is added, the tube is capped, weighed, the grip is changed and finally loaded into the shaker to be agitated for 45 minutes.
   e) The tube is removed from the shaker and loaded into a holder in front of the centrifuge.
   f) A balance tube is removed from the centrifuge and loaded into a second holder in front of the centrifuge.
   g) The sample tube is removed from the first holder and loaded into the centrifuge where a 3 minute spin is now initiated.
   h) While spinning, the robotic arm will perform a number of tasks:
      i) remove a gravity slip cap from a holding tube
      ii) remove a holding tube from it's rack.
      iii) weight the empty holding tube
      iv) load the holding tube into the prep-sep workstation
      v) change heads
      vi) remove a prep-sep cartridge from the prep-sep dispenser
      vii) load the prep-sep cartridge into the prep-sep workstation
      viii) wash the prep-sep cartridge
      ix) change heads
      x) wait for the spin to complete

7. When the spin is complete, the arm will:
   a) unload the sample tube from the centrifuge into the first holder
   b) re-load the balance tube from the second position into the centrifuge
   c) remove the sample tube from the first holder
   d) uncap the sample tube
   e) place the sample tube in the balance and store it's weight uncapped
   f) leave the tube in the balance and pickup a cannula hand
   g) position the cannula over the sample tube
   h) find the surface of the solvent phase
   i) calculate an offset from the surface such that at least 90% of the extraction solvent is removed
   j) draw the solvent back into a 25 ml syringe on the MLS
   k) position the cannula over the prep-sep in the prep-sep station
   l) dispense the solvent into the prep-sep cartridge
   m) force the solvent thru the prep-sep packing into the holding tube.
   n) remove the holding tube from the prep-sep station and return it to it's rack

o) wash the cannula and syringe. Dispose of the used prep-sep
p) cap the holding tube with a gravity slip cap
q) prefill a syringe with a second extraction solvent portion
r) remove the tube from the balance and dispense the second solvent portion into the tube
s) cap the tube, shift the grip and load it into the shaker, where it will agitate again for 45 minutes

8. Upon completion of the second agitation, steps 3, 4, 5, 7 (a)-(j) are repeated.

9. The robot will then continue operations as follows:
   a) dispose of the used prep-sep cartridge
   b) remove the holding tube from the prep-sep workstation and load it into the vortexer
   c) vortex the combined extracts for 2 minutes

10. While vortexing, the following steps will be performed:
   a) the robotic arm will unload the sample tube from the balance
   b) the contents are aspirated to a waste reservoir
   c) the sample tube is capped and returned to it's rack

11. When vortexing is complete, the following sequence will occur:
   a) unload the holding tube from the vortexer and load it into the balance
   b) record the weight of the tube and the combined extracts
   c) pickup and position the cannula tip to 0.5 cm above the surface of the combined extracts.
   d) find the liquid surface
   e) calculate an offset
   f) draw a 1 g aliquot of solvent into the 25 ml syringe
   g) dispense this 1 g aliquot to an output tube
   h) wash the cannula and syringe
   i) remove the holding tube from the balance and replace it in the rack
   j) cap the holding tube with a gravity slip cap

This outline lists the robotic steps performed on a sample in a sequential manner. However, to use each work station to it's greatest potential (i.e. minimize idle time) the system will be required to run in a serialized mode. This procedure requires that modules such as the shaker and centrifuge operate independantly of the arm, so in effect the system idle time is minimized. This was accomplished by fine-tuning the timing of each step so that synchronization between the arm and the modules was achieved.

In addition, at critical steps such as capping and getting a pippette tip, confirm operations are carried out to ensure the proceeding step was successful before the subsequent operation is carried out.

SERIALIZATION OF THE ROBOTIC PROCEDURE

From the documention supplied by Zymark, an algorithm was devised to serialize this application. The basic method is defined as follows:

Robotic Step: an activity where the arm is utilized, i.e.: capping tubes, moving tubes around the table.

Non-Robotic Step: an activity where the arm is <u>not</u> utilized, i.e.: a tube being agitated, spun in the centrifuge.

In this development work, the robotic control program is divided into sections, terminated by a non-robotic step and the times to complete these sections are obtained. For example:

Section I	-	Get sample tube	0.8 min
	-	Load into shaker	
Section II	-	Add first portion of extraction solvent	4 min
	-	Load shaker	
Section III	-	Unload shaker	17 min
	-	Centrifuge sample	
	-	Pipette off solvent phase	
	-	Add second extraction solvent portion	
	-	Load shaker	
Section IV	-	Unload shaker	21 min
	-	Centrifuge sample	
	-	Pipette of second solvent phase	
	-	Aspirate remaining contents to waste receptacle	
	-	take 1 g aliquot	
			42.8 min

Therefore Total Robotic Time/Sample = 42.8 min = .7 hours

Therefore Sample Output Rate = $\frac{1 \text{ sample}}{.7 \text{ hours}}$ x 24 hours = 33 sample/day

To minimize programming complexity, centrifuge, although by definition a non-robotic step is turned into a robotic step by utilizing the arm while the sample is spinning, i.e.: by setting up the prep-sep station.

Since the robotic time per sample is fairly close to the 1 hour sample agitation time, the procedure is time-balanced against robotic time, rather than agitation time. If the procedure were balanced against a strict requirement for a 1 hour agitation time, there would be a dead time of 17.2 minute/sample, which implies a drastic reduction in throughput.

However, this change in time could only be justified if it had no adverse effect on recoveries, which was tested by running spiked and real samples through the system.

SOFTWARE

A major limitation of the Zymate II system is it's 60K memory for the system dictionary, programs and data. A memory upgrade is not cost-effective since contract specifications called for a PC driven system anyway. To accomplish this, an IBM compatible P.C. was linked to the controller to provide:

i. a menu-driven user interface for the system.
ii. expanded data storage and processing capabilities to the system.
iii. enhanced error-recovery.
iv. uploading of data to other systems.

This way the controller functions as a transparent low-level module, while the P.C. functions as a high-level controller, responsible for data reductions, file manipulation and repeat production.

During operation, the operator is presented with a main menu from which he or she can access to all phases of the robotic procedures. The system is broken into two phases:

1) Pre-digestion Routines
2) Extraction Routines

In the pre-digestion routine, the operator is presented with a form where he enters date, name, method, LIS # and workstation. He is then requested to enter the number of samples he wishes to digest. Sample attributes are entered next, in such a way that the number of keystrokes is minimized, i.e. with only sample codes. Upon completion, the empty tubes are first weighed and stored in a data file. The operator will then load the samples into the tubes. The rest of the procedures will be handled by the robotic system.

In the extraction routine, data entries and manual intervention are minimal. In the beginning of the run, the operator will review a checklist shown on the terminal to ensure the robot has sufficient supply of disposable sep-pak cartridges, solvents and reagents for the duration of the run. The rest of the operation again will be automatic.

EVALUATION OF THE ROBOTIC SYSTEM AND PROGRAMMING CONSIDERATIONS

During the course of the method development, a number of problems were encountered when trying to convert the manual method to the robotic method. Some examples are presented below:

(i) one of the contract specifications required was that all materials to be in contact with the extracts were to be made of stainless or Teflon. However, the Teflon coated caps for the 40 ml centrifuge

tubes were found to leak excessively when loaded on their sides in the linear shaker. Increasing capping torque had the effect of overtightening the caps such that they could not be uncapped by the arm. To solve this problem, extensive modification of the existing capping software was carried out to eliminate capping error. In these procedures, the robotic hand would test the torque of the cap. If the required torque could not be achieved, the robot would try two more times to tighten the cap and check for leaking by the weight difference.

(ii) Corrosion in the system was severe among metallic parts in contact with acid. In this work, the metal feed tubes in the dispensing post were removed and replaced with a Teflon tubing to eliminate this problem. However, cannular tip was also found to be corroded and consequently led to a loss of pipetting efficiency. Valves in contact with acid and solvent had to be frequently replaced during our development process.

(iii) The Zymark centrifuge consisted of rubber bungs in the bottom of the bucket. After centrifugation of samples, vaporized solvent would stick onto the rubber, forming good seal with the bottom of the centrifuge tubes. After a spin, the bucket would stay attached and be pulled out of the centrifuge which would lead to a collison. The solution was to insert a Teflon coated septum of proper diameter into the bucket , which eliminate the bonding of the rubber with the tubes.

(iv) Because there is an element of potential mis-alignment in the system, (e.g. racks, the shaker, centrifuge buckets, balance tube holders etc. where tubes can move slightly,), it was necessary to develop and incorporate checking steps into all routines which will affect throughput slightly, but result in less failures.

(v) Initial design included the use of 10 ml disposable plastic pipette tips to withdraw the upper layer of solvent from a centrifuge tube. However, due to variations in tube volume and sample size, the surface level of the extraction solvent will vary. Thus, the routine to find the initial starting point at which to begin a pipette operation is necessary. It was also discovered that the pipette tips are not perfectly straight and so may touch the wall of the tube. This results in an increasing weight reading during pipetting. A cannula tip was then subsequently used, which solved the pipetting problem and gave precise weights. However, this required a wash station of it's own and addition steps for transferring.

(vi) Depending on the type of fish, emulsions did form in some samples at high shaker speeds, which could not be broken by the centrifuge in the time alloted. Adjustments were made to shaker speed, centrifuge speed, to minimize this problem.

(vii) Pipetting more than 95% of solvent from top of acid phase was found to be very difficult. For this reason, the samples was extracted at least two times, which minimize the loss of solvent. In addition, several checking steps were incorporated into the pipetting software to compensate this problem.

(viii) Variations in glassware size required extensive sample abort programming, due to the probability of failure in an operation. Theoretically, every move should be confirmed on systems such as this. However, this would also increase the complexity of the software, decrease the maintainability of the completed code and fall outside the time constraints of the project. Compromises were therefore made

in the design of the software; operations with a high probability of failure, i.e.: capping were extensively confirmed; operations with a low probability on the other hand, were not as in the case of vortexer operations.

(ix) Variations in the sample matrix (fish tissue) e.g. water content and lipid content, required some development of an appropriate pipetting algorithm. Attempts were made to define cannula pipette tip position over the acid/solvent interface as a function of sample weight. The interface level, however, varied too much for this approach to be of any practical use. By assuming negligible miscibility of acid and solvent conbined with a top-down approach (quite literally in this case!), an algorithm was developed with the accuracy and reproducibility surpassing that of a manual method. An optical sensor approach to measure interface height was also considered, but abandoned upon advice from Zymark, who had previous experience with these systems.

(x) Initial runs had low recoveries due to a discreprency in the robotic specifications and the manual method. All manually extracted samples were allowed to digest overnight, whereas the robotic method explicitly stated 1 hour digestion time. This necessitated a major redesign and rewrite of the completed code

The final phase of this project involved a full and comprehensive analytical evaluation of the completed robot extraction workstation. This evaluation included extraction of 50 samples of each of:

1) Blanks (reagent only, no biological tissue present)
2) Spikes (fortified samples as per MOE technique)
3) Duplicates (multiple extractions of same tissue homogenate).

All the extractions have so far been completed, however, at the point of writing, the complete data are not fully avaliable. Since improved precision was one of our expectations of robotic automation, we had initially carried out a number of experiments to evaluate the reproducibility of the system. The results from these experiments are presented in Table I. Table II also tabulates the results of a comparison study of spiked fish samples between the manual and robotic method. The manual extraction and the final gas chromatographic analysis of the extracts were conducted in the MOE laboratory to give an accurate comparison of the developed robotic method and the manual method currently used in MOE laboratories. Review of the data indicates that the robotic method is as good or better than the manual method. In terms of reproducibility, the results are quite satisfactory.

CONCLUSION

In summary, the robotic workstation developed for the extraction of the fish tissues has demonstrated sufficient accuracy, precision and reliability as compared to the manual method. Although it is a complicated laboratory robotic application, statistical evaluation reveals that the method is quite reproducible and the overall sample throughput surpassing the requirement set by the Ministry of the Environment (33 samples/day).

Table I

COMPOUND	ACTUAL STD. CONC.	DETECTED BLANK SPIKE CONC.	MEAN DETECTED CONC. *	OVERALL % REC. **	STANDARD DEVIATION	C.V. %
PCB	1000	997.50	989.40	98.90	82.90	8.40
HCB	10	10.50	13.80	137.00	2.60	19.10
HEPTACHLOR	20	21.50	22.70	113.50	3.10	13.80
ALDRIN	40	49.50	45.10	112.80	5.00	11.00
PP-DDE	90	108.00	94.20	104.70	16.00	17.00
MIREX	100	138.50	146.40	146.40	33.50	22.80
ALPHA-BHC	40	25.80	38.80	97.00	7.60	19.80
BETA-BHC	40	33.80	46.70	116.80	8.20	17.60
GAMMA-BHC	40	34.50	48.70	121.80	8.70	17.80
ALPHA-CHLORDANE	40	36.80	40.20	100.50	3.93	9.80
GAMMA-CHLORDANE	40	32.30	34.80	87.00	3.22	9.20
OP-DDT	40	27.50	27.80	69.50	6.20	22.30
PP-DDD	40	33.50	37.70	94.30	4.55	12.10
PP-DDT	40	35.00	32.70	81.80	6.40	19.50
OCTACHLOROSTYRENE	20	21.30	22.30	111.50	4.07	18.30
P1BHCD	40	34.00	35.20	88.00	6.098	17.30
P1CHLN	40	35.80	34.30	85.50	7.62	22.20

Table II

COMPOUND	MEAN		STANDARD DEVIATION		C.V.(%)	
	MANUAL	ROBOTIC	MANUAL	ROBOTIC	MANUAL	ROBOTIC
PCB	911.40	989.40	148.80	82.90	16.30	8.40
HCB	16.90	13.80	7.30	2.60	43.30	19.10
HEPTACHLOR	19.90	22.70	4.90	3.10	24.50	13.80
ALDRIN	47.70	45.10	7.70	5.00	16.20	11.00
PP-DDE	133.60	94.20	21.20	16.00	15.90	17.00
MIREX	130.90	146.40	17.40	33.50	13.30	22.80
ALPHA-BHC	20.00	38.80	6.20	7.60	31.10	19.80
BETA-BHC	37.70	46.70	10.00	8.20	26.50	17.60
GAMMA-BHC	31.00	48.70	8.80	8.70	28.40	17.80
ALPHA-CHLORDANE	27.20	40.20	6.80	3.93	24.90	9.80
GAMMA-CHLORDANE	26.20	34.80	6.70	3.22	25.70	9.20
OP-DDT	18.90	27.80	10.40	6.20	54.70	22.30
PP-DDD	25.50	37.70	6.30	4.55	24.90	12.10
PP-DDT	23.20	32.70	5.60	6.40	23.90	19.50
OCTACHLOROSTYRENE	28.30	22.30	7.80	4.07	27.50	18.30
P1BHCD	N/A	35.20	N/A	6.10	N/A	17.30
P1CHLN	N/A	34.30	N/A	7.62	N/A	22.20